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ENTEROVIRUS, ARBOVIRUS AND RMSF SEASON

TAXONOMIC AND NOMENCLATURAL CHANGES IN Enterobacteriaceae - Part I

ENTEROVIRUS, ARBOVIRUS AND RMSF SEASON

In Montana, spring and summer bring seasonal upswings in the incidence of certain arthropod-borne viral and rickettsial diseases, including Western Equine Encephalitis (WEE), Colorado Tick Fever (CTF) and Rocky Mountain Spotted Fever (RMSF). Therefore, when encephalitides, exanthema and fevers appear during these seasons, the arthropod-borne agents are often the first to be indicted.

Often overlooked in diagnosis is a far more prevalent group of etiologic agents, the enteroviruses of the family Picornaviridae (Coxsackieviruses, Echoviruses and Polioviruses). These viruses, rather than being primarily agents of acute gastroenteritis, commonly cause a broad range of diseases, including "aseptic" meningitis, meningoencephalitis, epidemic pleurodynia (Bornholm Disease), myocarditis, pericarditis, herpangina and occasional exanthematous diseases including hand, foot and mouth syndrome. Thus, the pathogenic effects of many enteroviruses may resemble those of the arthropod-borne agents, presenting a problem in clinical and laboratory diagnosis.

Specimens for Diagnosis

Serologic specimens are not useful for diagnosis of enteroviral diseases; virus isolation from throat specimens (within 3 days after onset) and stools (1-3 weeks after onset) should be attempted instead. Conversely, WEE, CTF and RMSF cannot be diagnosed from throat and rectal swabs; acute and convalescent phase sera are required.

Therefore, when encephalitides, questionable exanthema and febrile illnesses are to be diagnosed, a battery of sera, throat swabs and rectal swabs (or stools) should be submitted. Swabs for virus isolation should be taken at or near the onset of disease. The acute phase serum should be drawn at onset and the convalescent specimen 2-3 weeks later.

Enteroviral illnesses often occur in familial or nonfamilial clusters, with several individuals showing similar symptoms within a short time span. Children are the most frequent target of enterovirus diseases. Enteroviruses are highly communicable, and generally spread horizontally via preschool children. Vertical transmission to nonimmune family members may also occur.

Arthropod-borne viral and rickettsial diseases occur among all age groups, usually as isolated cases. These diseases are communicated to man only through the bite of an infected vector, and direct person to person spread does not occur.

TAXONOMIC AND NOMENCLATURAL CHANGES IN Enterobacteriaceae - Part I

The Center for Disease Control (CDC) recently announced some significant changes in taxonomy and nomenclature in Enterobacteriaceae.(1) Some of these changes have already been incorporated into commercial identification schemes (e.g. Enterobacter gergoviae in the API system), even though many reference laboratories, including ours, have until now not recognized these changes. Other changes (e.g. Hafnia alvei) have been in use for some time.

The purpose of this note is to acquaint laboratories in Montana with many of the changes proposed by CDC. We have incorporated these changes into our own reference work scheme. Workers at CDC have also proposed major taxonomic changes in the tribe Proteeae which will be the subject of Part II of this report in a future Laboratory Bulletin.

The changes discussed herein are listed in Table I. See Page 5.

1. Klebsiella oxytoca. This species previously was identified as Klebsiella pneumoniae (indole positive). Genetic studies have shown that these organisms form a distinct genetic as well as biochemical group different from K. pneumoniae. Identification by routine testing is simple, since their reactions are typical of K. pneumoniae, except that they are indole positive. Other characteristics of interest in this species include a variable gelatin reaction and pectate hydrolysis (when tested by the method of Von Riesen).

2. Enterobacter sakazakii. Biochemically, these organisms resemble Enterobacter cloacae but can readily be distinguished by:

A) Yellow pigment (Trypticase soy agar @ 25°C 1-3 day incubation).

B) No acid produced from Sorbitol.

C) DNase positive (3-6 days on DNase agar plus toluidine blue @ 36°C).

3. Enterobacter gergoviae. These organisms most closely resemble E. aerogenes in their biochemical reactions, but can be routinely differentiated by a number of characteristics, including:

A) Urease positive B) Sorbitol negative C) KCN negative

4. Hafnia alvei. Biochemical and genetic studies have confirmed that organisms formerly classified as Enterobacter hafniae should be placed in a separate genus. At present, only one species, H. alvei, is recognized. The biochemical characteristics used to differentiate E. hafniae (2) are sufficient to identify Hafnia alvei. Laboratories should be aware, however, that this is a biochemically and genetically diverse species which will probably be divided into new species in the future.

5. Citrobacter amalonaticus. For some time, it has been obvious that recognition of only two species of Citrobacter has been, at best, unwieldy. The attempt to move groups of Citrobacter to the proposed genus Levinea has not met with overwhelming acceptance. This species, C. amalonaticus, was previously included as a biogroup of C. freundii, from which it differs extensively. Differential tests for Citrobacter species include:

	H_2S	Indole	Malonate	KCN
C. freundi	+	-	-	+
C. diversus	-	+	+	-
C. amalonaticus	-	+	-	+

6. Yersinia enterocolitica. With the increasing awareness of Yersinia enterocolitica as a human pathogen and the increasing frequency of its isolation from clinical specimens, much effort has gone into re-evaluation of the classification of this organism. Strains of Y. enterocolitica can be quite variable biochemically, which has led to three major schemes of biotyping. Correlation of biotypes with DNA hybridization studies has indicated that it is possible to assign isolates to DNA relatedness groups by some key biochemical reactions (Table 2). At the present time we will continue to report isolates in all four of the Y. enterocolitica DNA relatedness groups as Y. enterocolitica. It appears likely, however, that the organisms in groups 2 and 3 will eventually be recognized as new species. We also recommend that laboratories not familiar with Y. enterocolitica review material on the isolation and identification of these organisms.(3) We have experienced a significant increase in the numbers of these organisms referred to our laboratory during the past year.

Table 2. Biochemical Characterization of Y. enterocolitica DNA Relatedness Groups (1)

Reaction	Relatedness Group 1	2	3	4
L-Rhamnose	-	+	+	-
Raffinose	-	+	-	-
Melibiose	-	+	-	-
alpha-methyl glucoside	-	+	-	-
Sucrose	+	+	+	-

+ = 90% or more positive

- = 10% or less positive

Laboratories wanting more detailed and extensive information about these changes and new species are urged to contact Dr. Douglas Abbott, Supervisor of Clinical Bacteriology, State Microbiology Laboratory, Capitol Station, Helena Montana 59601, (406) 449-2642

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2. Edwards, P. R. and W. H. Ewing. 1972. Identification of Enterobacteriacea. Burgess Publishing Company, Minneapolis

3. Lennette, E.H., E.H. Spaulding, and J. P. Truant. 1974. Manual of Clinical Microbiology. American Society for Microbiology, Washington, D. C.

MICHAEL R. SKEELS, Ph.D., Editor

TABLE I - Proposed changes in Taxonomy and Nomenclature (1)

New Designation	Previous Designation
Klebsiella oxytoca	*Klebsiella pneumoniae* (indole positive Biogroup)
Enterobacter sakazakii	*Enterobacter cloacae* (yellow pigment)
Enterobacter gergoviae	
Hafnia alvei	*Enterobacter hafniae*
Citrobacter amalonaticus	*Citrobacter freundii* (H_2S negative, malonate negative, indole positive, adonitol positive biogroup)
Yersinia enterocolitica (typical)	*Y. enterocolitica*
Y. enterocolitica (sucrose negative)	*Y. enterocolitica*
Y. enterocolitica (rhamnose positive)	*Y. enterocolitica*
Y. enterocolitica (rhamnose and raffinose positive)	*Y. enterocolitica*
**Providencia stuartii*, urea positive	*Proteus rettgeri*, Biogroup 5
**Providencia stuartii* Biogroup 4	*Providencia alcalifaciens* Biogroup 4
**Providencia rettgeri*	*Proteus rettgeri*, Biogroups 1-4
**Morganella morganii*	*Proteus morganii*

* Details of these changes will be described in a future Laboratory Bulletin.

STATE DEPARTMENT OF HEALTH
AND ENVIRONMENTAL SCIENCES
HELENA, MONTANA 59601
LABORATORY DIVISION